

Relation Between Sex, Menopause, and White Matter Hyperintensities

The Rhineland Study

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Abstract

Background and Objectives

Mounting evidence implies that there are sex differences in white matter hyperintensity (WMH) burden in older people. Questions remain regarding possible differences in WMH burden between men and women of younger age, sex-specific age trajectories and effects of (un)controlled hypertension, and the effect of menopause on WMH. Therefore, our aim was to investigate these sex differences and age dependencies in WMH load across the adult life span and to examine the effect of menopause.

Methods

This cross-sectional analysis was based on participants of the population-based Rhineland Study (30–95 years) who underwent brain MRI. We automatically quantified WMH using T1-weighted, T2-weighted, and fluid-attenuated inversion recovery images. Menopausal status was self-reported. We examined associations of sex and menopause with WMH load (logit-transformed and z-standardized) using linear regression models while adjusting for age, age-squared, and vascular risk factors. We checked for an age × sex and (un)controlled hypertension × sex interaction and stratified for menopausal status comparing men with premenopausal women (persons aged 59 years or younger), men with postmenopausal women (persons aged 45 years or older), and premenopausal with postmenopausal women (age range 45–59 years).

Results

Of 3,410 participants with a mean age of 54.3 years ($SD = 13.7$), 1,973 (57.9%) were women, of which 1,167 (59.1%) were postmenopausal. We found that the increase in WMH load accelerates with age and in a sex-dependent way. Premenopausal women and men of similar age did not differ in WMH burden. WMH burden was higher and accelerated faster in postmenopausal women compared with men of similar age. In addition, we observed changes related to menopause, in that postmenopausal women had more WMH than premenopausal women of similar age. Women with uncontrolled hypertension had a higher WMH burden compared with men, which was unrelated to menopausal status.

Discussion

After menopause, women displayed a higher burden of WMH than contemporary premenopausal women and men and an accelerated increase in WMH. Sex-specific effects of uncontrolled hypertension on WMH were not related to menopause. Further studies are warranted to investigate menopause-related physiologic changes that may inform on causal mechanisms involved in cerebral small vessel disease progression.

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Glossary

3D = 3-dimensional; BMI = body mass index; FLAIR = fluid-attenuated inversion recovery; FOV = field-of-view; HT = hormone therapy; IQR = interquartile range; TE = echo time; TI = inversion time; TR = repetition time; WMH = white matter hyperintensities.

White matter hyperintensities (WMH) have been associated with distinct neurologic symptoms including stroke,¹ motor^{2,3} and mood disturbances,^{4,5} and cognitive dysfunction.^{1,6,7} Previous studies investigating sex differences in WMH burden have reported inconsistent results. Although studies in the older individuals mainly found that women exhibit higher levels and faster progression of WMH burden,⁸⁻¹⁰ a study in middle-aged and older individuals found that men have more WMH than women.¹¹ Although studies typically adjust for sex differences in WMH, the underlying sex-specific mechanisms remain poorly understood.

Menopause is a key event in a woman's life, and age at natural menopause has been associated with cardiovascular disease,¹² dementia,¹³ stroke,¹⁴ and all-cause mortality.^{15,16} In addition, it has been suggested that postmenopausal status is associated with higher WMH burden in women.^{11,17} What remains unclear, however, is whether sex is associated with WMH burden at younger ages, if there are sex-specific age trajectories, and whether menopausal status underlies later-life sex differences.

Hypertension, especially when uncontrolled, is a main risk factor for WMH.¹⁸⁻²⁰ Whereas sex differences in hypertension are recognized,²¹ it is unknown whether the effect of hypertension on WMH burden differs by sex and menopausal status.

In this study, we aimed to examine the extent to which sex differences exist in WMH load across the adult life span in a population-based cohort. Specifically, we determined whether these sex differences are modified by menopause. In addition, we investigated sex-specific age trajectories and effects of hypertension on WMH.

Methods

Study Population

This study is based on the first 5,000 consecutive participants enrolled in the Rhineland Study. The Rhineland Study is an ongoing, prospective, single-center, community-based cohort study. All residents aged 30–95 years from 2 geographically defined areas in Bonn, Germany, are invited to participate. The sole exclusion criterion for enrolment is insufficient German language proficiency or lack of mental capacity to provide signed informed consent. Participants who had active implants (e.g., pacemakers), passive medical devices of which we could not confirm MRI eligibility, intrauterine devices, nonmedical metal and metal splinters, or were pregnant were

excluded from the MRI examination because of MRI contraindications.

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the Ethics Committee of the University of Bonn, Faculty of Medicine, and is conducted in accordance with the recommendations of the International Council for Harmonization Good Clinical Practice. At the time of enrolment, we obtained written informed consent from all participants in accordance with the Declaration of Helsinki.

MRI

MRI data were acquired on 3 Tesla MRI scanners (Siemens Prisma Magnetom, Erlangen, Germany) equipped with an 80 mT/m gradient system and a 64-channel phased-array head-neck coil. The protocol included the following sequences: a 3-dimensional (3D) T1-weighted multiecho magnetization prepared rapid gradient-echo sequence (acquisition time [TA] = 6.5 minutes, repetition time [TR] = 2,560 msec, inversion time [TI] = 1,100 msec, flip angle 7°, field-of-view [FOV] = 256 × 256 mm, 0.8 mm isotropic),^{22,23} a 3D T2-weighted turbo-spin-echo sequence (TA = 4.6 minutes, TR = 2,800 msec, echo time [TE] = 405 msec, FOV = 256 × 256 mm, 0.8 mm isotropic),^{24,25} and a 3D T2 fluid-attenuated inversion recovery (FLAIR) sequence (TA = 4.5 minutes, TR = 5,000 msec, TE = 393 msec, TI = 1,800 msec, FOV = 256 × 256 mm, 1.0 mm isotropic). All sequences use twofold parallel imaging acceleration using CAIPIRINHA and elliptical sampling.^{26,27}

Assessment of WMH

We defined WMH as hyperintense signals in the white matter tracts on T2-weighted images (see eMethods, links.lww.com/WNL/C85).²⁸ In brief, we automatically outlined WMH using an in-house developed pipeline using DeepMedic,²⁹ based on image information from the T1-weighted, T2-weighted, and FLAIR sequences. The algorithm was trained on 30 and tested on 10 images, which were manually segmented by 1 rater, and visually quality controlled by an experienced neuroscientist. To ensure the quality of the automated WMH segmentation, we manually assessed a subset of 908 participants and additionally excluded 110 participants with other pathology present (e.g., stroke, multiple sclerosis), 23 because of insufficient image quality, and 20 because of pipeline failures. White matter volume was extracted using FreeSurfer's automated segmentation.³⁰

Sex, Menopause, and Covariates

Sex refers to biological sex, with women being biological female and men biological male at birth. Menopause was

assessed as status (yes/no) at baseline examination and was self-reported. Women who indicated they underwent bilateral oophorectomy or had no menstruation for more than a year not due to pregnancy, breastfeeding, or contraception, and women older than 60 years were classified as postmenopausal. We excluded women who underwent hysterectomy without bilateral oophorectomy because their menopausal status could not be determined. We defined hypertension as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or antihypertensive medication use; we thereby distinguished between controlled hypertensive participants who had systolic blood pressure < 140 mm Hg and diastolic blood pressure < 90 mm Hg while using regularly antihypertensive medication and uncontrolled hypertensive participants who had systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg. Diabetes was defined as fasting plasma glucose level ≥ 7 mmol/L, hemoglobin A1c $\geq 6.5\%$, or use of antidiabetic medication. History of coronary heart disease (angina pectoris, myocardial infarction, coronary bypass operation, coronary artery stenting), valve disease, intermittent claudication, heart failure, and arrhythmia was self-reported and summarized as prevalent cardiovascular disease. Smoking status was obtained from a self-reported questionnaire, classified as current or nonsmoker. Missing smoking status ($n = 197$) was imputed based on the levels of the nicotine metabolite cotinine in blood, which were measured by the Metabolon HD4 platform.³¹ We set the 97.5th percentile of cotinine levels in nonsmokers as a cut-off value. Based on this, we then assigned the participants as either current smokers or nonsmokers. Other covariates were body mass index (BMI) and use of lipid lowering medication. Education was defined as the highest, self-reported educational attainment and categorized based on the International Standard Classification of Education 2011 as low (lower or secondary education), middle (completed secondary education up to completed Bachelor's degree or equivalent), or high (completed Master's degree, equivalent, or higher).³² The use of hormone therapy (HT) in postmenopausal women was assessed based on the Anatomical Therapeutic Chemical code of the self-reported medication.

Statistical Analysis

WMH lesion load was calculated as WMH volume divided by white matter volume to account for brain atrophy. We logit-transformed WMH load because of its skewness and z-standardized it before further analysis. We assessed differences in WMH load between men and women using linear multivariable regression. All models were adjusted for age (mean-centered), sex, and vascular risk factors (hypertension, diabetes, prevalent cardiovascular disease, smoking, body mass load index, and use of lipid-lowering medication). In addition, we checked for nonlinear relationships with age by including age-squared as an independent variable and for interactions between age and sex and (un)controlled hypertension and sex by including an age \times sex and (un)controlled hypertension \times sex interaction term to the models.

First, we tested for overall sex differences in WMH between men and women. Next, we stratified for menopausal status. In our study population, premenopausal women were between load age 30 and 59 years and postmenopausal women between age 41 and 95 years. In this stratification, we excluded postmenopausal women younger than 45 years because they experienced early menopause ($n = 7$); therefore, postmenopausal women in the stratification were between age 45 and 95 years. We thus compared lesion load between (1) men (reference group) and premenopausal women (persons aged 59 years or younger), (2) premenopausal (reference group) and postmenopausal women (age range 45–59 years), and (3) men (reference group) with postmenopausal women (persons aged 45 years or older). Here, we adjusted the models for age, vascular risk factors, sex (models 1 and 3), menopause (model 2), and age-squared (model 3). Finally, we examined whether WMH load differed between postmenopausal women who did or did not receive HT.

All analyses were performed in R version 4.0.2,³³ and p values less than 0.05 were considered statistically significant. Missing covariates were imputed based on nonparametric missing value imputation applying random forest using the R package missForest (version 1.4).³⁴

Data Availability

The data for this manuscript are not publicly available because of data protection regulations. Access to data can be provided to scientists in accordance with the Rhineland Study's Data Use and Access Policy. Requests for additional information and/or access to the data sets can be send to RS-DUAC@dzne.de.

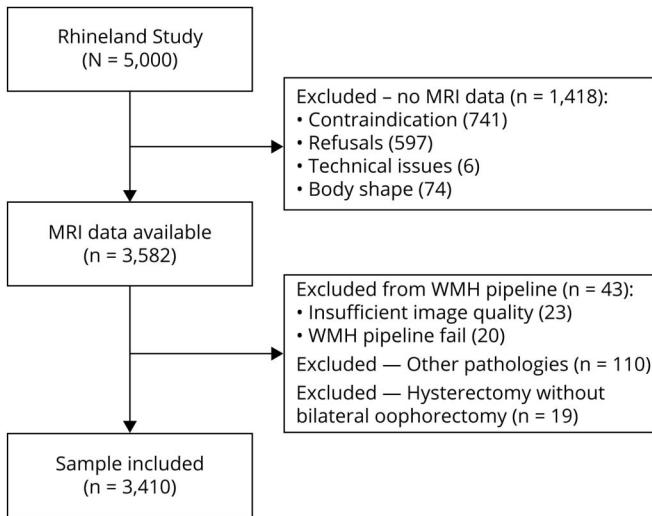
Results

Characteristics of the Study Population

Selection and characteristics of our study population are presented in Figure 1 and Table 1, respectively. The mean age of the 3,410 participants included in this study was 54.3 ± 13.7 years, and 1,973 (57.9%) were women. Of the women, 1,167 (59.1%) were postmenopausal, of whom 216 (18.5%) were on HT. Hypertension was present in 1,208 (35.4%) participants, of whom 660 (54.6%) had uncontrolled hypertension. Participants for whom no MRI was available or where the MRI did not pass quality assurance were on average significantly older, less educated, more often male, and had a higher BMI and more often hypertension or prevalent cardiovascular disease. In the 45–59 years age range, postmenopausal women were older than premenopausal women and had a higher BMI and lower education.

Median WMH volume in the whole cohort was 0.5 mL (interquartile range [IQR] 0.2–1.2 mL), and median WMH load was 0.1% (IQR 0.1%–0.3%). Table 2 presents the WMH burden characteristics of the study population and the subgroups stratified by sex and menopausal status.

Figure 1 Cohort Selection



Body shape: Participants who could not be placed inside the MRI because of size of head, shoulder, or waist circumference being too big for the scanner. WMH = white matter hyperintensity.

Overall Sex Differences in WMH

Figure 2A shows the WMH burden stratified by sex within our population. We saw that with increasing age WMH load increased exponentially and that sex effects changed over the age span (age \times sex interaction: $p < 0.001$), with sex differences becoming more pronounced after menopause.

Age effects were stronger in women than in men (age, b per year increase = 0.05 [95% CI 0.04–0.05]; nonlinear age dependency age-squared, b per 10^{-2} year 2 = 0.06 [95% CI 0.04–0.07]; and age, b per year increase = 0.04 [95% CI 0.04–0.04]; nonlinear age dependency age-squared, b per 10^{-2} year 2 = 0.05 [95% CI 0.03–0.07], for women and men, respectively).

Stratification by Menopausal Status

Figure 2, B–D shows WMH burden across the subgroups.

Subgroup analysis showed that premenopausal women and men until age 59 years did not differ in WMH load and that WMH load increased linearly with age (b per year increase = 0.03 [95% CI 0.02–0.03]).

Postmenopausal women, however, had a higher WMH load compared with men of similar age. Increase in WMH burden accelerated with age, which was different for men and women (age \times sex interaction: $p = 0.03$). Nonlinear age effects were stronger in women (age, b per year increase = 0.05 [95% CI 0.05–0.06]; nonlinear age dependency age-squared, b per 10^{-2} year 2 = 0.07 [95% CI 0.02–0.12]) compared with men (age, b per year increase = 0.05 [95% CI 0.04–0.05]; nonlinear age dependency age-squared, b per 10^{-2} year 2 = 0.05 [95% CI 0.00–0.09]).

Postmenopausal women had also a higher WMH burden compared with premenopausal women of the same age range

($b = 0.21$ [95% CI 0.07–0.35]). WMH burden increased linearly with age for both premenopausal and postmenopausal women aged 45–59 years (b per year increase = 0.02 [95% CI 0.01–0.04]).

Effects of Uncontrolled and Controlled Hypertension on WMH Burden in Overall Population

Participants with controlled and uncontrolled hypertension had more WMH than normotensive participants, which was dependent on sex (sex \times uncontrolled hypertension interaction: $p < 0.005$). This association was stronger in women (controlled hypertension, $b = 0.15$ [95% CI 0.05–0.25]; uncontrolled hypertension, $b = 0.30$ [95% CI 0.21–0.40]) than in men (controlled hypertension, $b = 0.12$ [95% CI 0.01–0.25]; uncontrolled hypertension, $b = 0.01$ [95% CI 0.00–0.21]).

Effects of Uncontrolled and Controlled Hypertension on WMH Burden Stratified by Menopausal Status

We found an interaction between sex \times uncontrolled hypertension in premenopausal women and men until age 59 years ($p = 0.02$). Uncontrolled hypertension compared with normotension was associated with more WMH in premenopausal women aged 30–59 years ($b = 0.31$ [95% CI 0.11–0.51]), but not in men ($b = 0.05$ [95% CI –0.08 to 0.18]).

In postmenopausal women and men aged 45 years or older, participants with controlled and uncontrolled hypertension had more WMH than normotensive participants, which was depending on sex (sex \times uncontrolled hypertension interaction, $p = 0.02$). This association was stronger in postmenopausal women (controlled hypertension, $b = 0.21$ [95% CI 0.08–0.33]; uncontrolled hypertension, $b = 0.31$ [95% CI 0.20–0.43]) than in men (controlled hypertension, $b = 0.13$ [95% CI 0.00–0.26]; uncontrolled hypertension, $b = 0.12$ [95% CI 0.01–0.24]).

Table 1 Characteristics of the Study Population

| Characteristics | Whole cohort (n = 5,000) | Participants included in the analyses | | | | Excluded participants (n = 1,590) | p Value ^c |
|---|-----------------------------|---------------------------------------|----------------------|--------------------|----------------------|--------------------------------------|----------------------|
| | | Overall (n = 3,410) | Women (n = 1,973) | Men (n = 1,437) | p Value ^b | | |
| Age, y | 55.1 (14.0) | 54.3 (13.7) | 54.6 (13.6) | 53.9 (13.9) | 0.412 | 56.9 (14.4) | <0.001 |
| Women | 2,824 (56.5) | 1,973 (57.9) | | | <0.001 | 851 (53.5) | 0.003 |
| Postmenopausal women^a | 1,640 (58.1) | | 1,167 (59.1) | | | 473 (55.6) | 0.004 |
| Postmenopausal HT^a | 279 (17.0) | | 216 (18.5) | | | 63 (13.3) | |
| Education | | | | | <0.001 | | <0.001 |
| High | 2,621 (52.9) | 1,889 (55.8) | 951 (48.7) | 938 (65.5) | | 732 (46.6) | |
| Middle | 2,232 (45.1) | 1,436 (42.4) | 955 (48.9) | 481 (33.6) | | 42 (2.7) | |
| Low | 101 (2.0) | 59 (1.7) | 47 (2.4) | 12 (0.8) | | 796 (50.7) | |
| BMI, kg/m²^a | 25.9 (4.5) | 25.6 (4.2) | 25.2 (4.6) | 26.1 (3.4) | <0.001 | 26.7 (5.2) | <0.001 |
| CVD | 960 (19.3) | 576 (16.9) | 339 (17.2) | 237 (16.5) | 0.011 | 384 (24.3) | <0.001 |
| Smoking^a | 621 (12.5) | 437 (12.8) | 239 (12.1) | 198 (13.8) | 0.091 | 184 (11.6) | 0.384 |
| Diabetes^a | 261 (5.4) | 149 (4.4) | 63 (3.2) | 86 (6.0) | <0.001 | 112 (7.0) | 0.015 |
| Hypertension^a | 1,867 (38.2) | 1,208 (35.4) | 648 (32.8) | 560 (39.0) | <0.001 | 659 (42.7) | 0.197 |
| Uncontrolled | 959 (51.4) | 660 (54.6) | 349 (50.4) | 311 (55.5) | <0.001 | 299 (42.7) | <0.001 |
| Use of lipid lowering medication^a | 598 (12.1) | 365 (10.7) | 171 (8.7) | 194 (13.5) | <0.001 | 233 (14.7) | 0.262 |

Abbreviations: BMI = body mass index; CVD = cardiovascular disease; HT = hormone therapy.

Data are the number of participants (percentages) or mean (SD). Participants were excluded if no MRI was available or if their MRI scan did not pass quality assurance.

^a Participants with missing data: menopause: n = 11 (0.4%); HT in postmenopausal women: n = 13 (4.7%); education: n = 46 (0.9%); BMI: n = 24 (0.5%); smoking: n = 16 (0.3%); diabetes: n = 156 (3.1%); hypertension: n = 110 (2.2%); use of lipid lowering medication: n = 75 (1.5%).

^b p values comparing women and men, adjusted for age where applicable.

^c p values comparing participants with and without MRI, adjusted for age and sex where applicable.

In premenopausal and postmenopausal women within the same age range, uncontrolled hypertension was associated with increased WMH load ($b = 0.31$ [95% CI 0.11–0.51]), regardless of menopausal status.

HT and WMH in Postmenopausal Women

There was no difference in WMH load between postmenopausal women using HT and postmenopausal women who did not ($b = 0.03$ [95% CI –0.08 to 0.15]).

Discussion

In this population-based cohort, we found that (1) the effect of sex on WMH load changes over the adult life span, (2) postmenopausal women have a higher WMH load compared with men as well as premenopausal women of the same age range, and (3) the increase in WMH burden accelerated with advanced age for both men and women, where the acceleration is faster in women.

Our results imply that WMH evolve differently for men and women, where menopause is a defining factor. In this population-based cohort, we showed that there was no

difference between premenopausal women and men of similar age with respect to WMH burden. Postmenopausal women had a higher WMH burden than men of similar age. This agrees with previous reports from the literature, where it has been shown that in the older participants, that is, predominantly postmenopausal women, women had more WMH than men.^{8–10,35–38} However, a study using UK Biobank data found that men have more WMH than women.¹¹ This study, however, also found that woman had larger total brain volume which is not only contradictory to other cohorts³⁹ but also to other studies using UK Biobank data.^{40,41} In addition, we showed that postmenopausal women also had more WMH compared with premenopausal women of the same age range, which agrees with previous smaller studies.^{11,17}

Moreover, with increasing age, the WMH burden in the brain exponentially increases for both men and women, suggesting nonlinear age dependencies need to be taken into account in future studies.

We found that participants with uncontrolled hypertension had a higher WMH burden than participants without or with controlled hypertension. This is in line with previous studies.^{18,20} In addition, we showed sex-specific differences in

Table 2 WMH Burden Characteristics of the Study Population and the Subgroups Used in Our Analysis Stratified by Sex and/or Menopausal Status

| | n | WMH volume (10^{-1} mL) | WMH load ($10^{-1}\%$ of WM) |
|-------------------------------|-------|----------------------------|-------------------------------|
| Whole study population | 3,410 | 5.1 (2.3–12.2) | 1.1 (0.5–2.7) |
| Women | 1,973 | 5.1 (2.2–12.6) | 1.2 (0.5–3.1) |
| Men | 1,437 | 5.0 (2.5–11.7) | 1.0 (0.5–2.5) |
| Subgroup ≤59 y | | | |
| Premenopausal women | 800 | 2.4 (1.3–4.5) | 0.6 (0.3–1.0) |
| Men | 932 | 3.4 (1.8–6.0) | 0.7 (0.4–1.2) |
| Subgroup 45 y or older | | | |
| Postmenopausal women | 1,167 | 9.4 (4.5–24.7) | 2.3 (1.1–6.1) |
| Men | 1,045 | 7.2 (3.5–15.0) | 1.5 (0.7–3.3) |
| Subgroup 45–59 y | | | |
| Premenopausal women | 310 | 3.3 (1.7–6.3) | 0.7 (0.4–1.4) |
| Postmenopausal women | 448 | 5.1 (2.6–9.7) | 1.2 (0.7–2.2) |

Abbreviations: IQR = interquartile range; WM = white matter; WMH = white matter hyperintensity.

Data represent median (IQR). In the subgroup 59 years or younger, we included only premenopausal women to men of the same age range, whereas in the subgroup 45 years or older, we included only postmenopausal women with men of the same age range.

the effect of uncontrolled and controlled hypertension on WMH burden, suggesting that sex differences, which are also underlying cardiovascular diseases, such as hypertension, are also contributing to the vascular burden in the brain. We found that especially women are susceptible to increased blood pressure and WMH burden, even in midlife. These sex-specific differences, however, were not related to menopause.

The effect of menopause on WMH burden suggests that women, after menopausal onset, become more susceptible to vascular changes and disease in the brain. Although the mechanisms that underlie these sex differences are still unclear, our findings agree with previous studies that proposed a protective nature of estrogen.^{42,43}

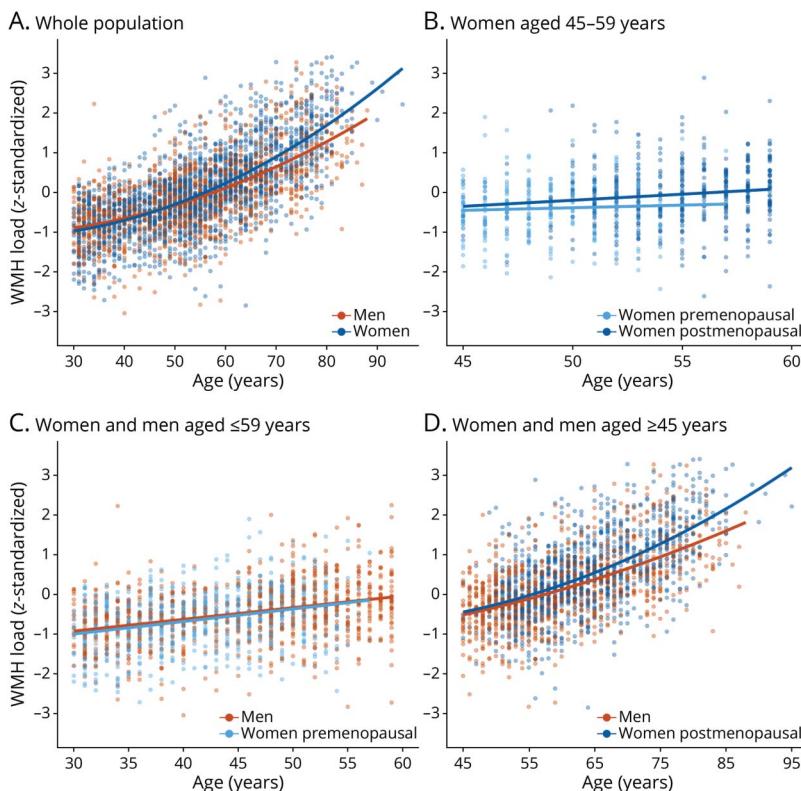
However, we observed no differences in WMH load in postmenopausal women using HT compared with those who did not, suggesting that HT after menopause does not continue this protective effect on the brain. This is supported by recent work that reported no preventive effect of HT with respect to the development of vascular dementia.⁴⁴

Menopause has been associated with physiologic changes beyond hormone levels. Reportedly, menopausal age is associated with methylation levels,⁴⁵ and an earlier onset of menopause has been associated with an increase in epigenetic age, a biological marker of accelerated aging.⁴⁶ Accelerated aging could thus be another mechanism explaining the increase in disease burden in women after menopause.

An alternative explanation for the relation with menopause may lie in the causes, rather than the consequences, of menopause. A recent study has identified loci that are associated with early or delayed onset of natural menopause, by engaging in the so-called DNA damage response (DDR).⁴⁷ The DDR is the primary biological pathway regulating age of menopause. Moreover, this study identified DDR pathways which were leading to cell death,⁴⁷ which might be the underlying mechanism explaining the increased WMH burden in postmenopausal women.

There are some limitations in our study. Our baseline questionnaire did not capture sex and gender identity of our participants in sufficient detail to account for the full and diverse spectrum.⁴⁸ We investigated biological sex differences in WMH burden, and we did not take into account gender differences. For example, biological female participants, who were assigned male or intersex at birth and used gender affirming hormones, may display different trajectories than the observations reported here. Therefore, our results cannot be generalized to a gender diverse population. Data on menopausal status were self-reported, and we did not have information on the age of menopausal onset or whether participants were perimenopausal. For the stratification in our analysis, we excluded postmenopausal women who were younger than 45 years to exclude women with early menopause. Because we did not ask for age at menopause, we cannot rule out that some older postmenopausal women had also experienced early menopause. Whereas we consider it unlikely that this has biased our findings, future research is required to further disentangle the effects of perimenopause.

Figure 2 WMH Burden in the Rhineland Study



(A) WMH load for men and women across age in the whole study population. (B) Differences in WMH load for premenopausal and postmenopausal women aged 45–59 years. (C) Differences between premenopausal women and men younger than 59 years. (D) Differences between postmenopausal women and men older than 45 years. WMH loads are logit-transformed and z-standardized. WMH = white matter hyperintensity.

and time of menopausal onset on WMH burden. In addition, we had no data on how long postmenopausal women had been using HT nor the type or dose, and the comparison between women with or without HT is limited by the small sample size. Furthermore, participants within the Rhineland Study cohort demonstrated a low burden of WMH and were in general quite healthy and well educated. Compared with the overall German population, the age and sex distribution of the Rhineland Study cohort shows the same distribution. However, participants of the Rhineland Study were more educated than the German population (high education: 52.9% compared with 18.5%) and were less likely to have diabetes (5.4% compared with 9.2%) or to smoke (12.5% compared with 22.4%). The prevalence of hypertension was higher in our cohort (38.2% compared with 31.6%), whereas the proportion of controlled hypertension was similar.⁴⁹ In addition, approximately one-third of the Rhineland Study cohort did not undergo MRI (Figure 1). These participants were less healthy than the ones who did undergo MRI. To the extent that this may have biased our estimates, we consider it most likely that it led to an underestimation of the true effects of sex and menopause on WMH burden rather than an overestimation.

Strengths of this work include the use of a large sample size drawn from a population-based cohort, which covered a

broad age range (30–95 years). This allowed us to examine overall sex differences in and specifically the effect of menopause on WMH comparing premenopausal women, postmenopausal women, and men of similar age, which before this study remained an open question. It is of importance that the study protocol includes comprehensive, standardized high spatial resolution neuroimaging data. The Rhineland Study is an ongoing, prospective study. Although this work presents cross-sectional baseline associations, the Rhineland Study has the potential to investigate the association between sex, menopause, and WMH burden longitudinally in the future. This is essential because both WMH and menopause are a manifestation of an aging process, with the latter being additionally associated with a deterioration in white matter health. A longitudinal future study comparing WMH progression between women with different ages of menopausal onset might shed light into the causal pathways underlying vascular brain health in women.

Within this large population-based cohort covering the adult life span, we identified sex differences in WMH which were dependent on menopausal status and showed that increase in WMH burden accelerates with age, especially for women. This highlights the need of sex-specific analyses to enhance our understanding of the disease burden. WMH are being investigated as biomarkers for disease and disease

outcome, for example, in stroke.¹ Our results demonstrate the necessity to account for different trajectories for men and women and menopausal status. This further underscores the importance of sex-specific medicine and the requirement for a more attentive therapy for older/postmenopausal women, especially with advanced vascular risk factors.

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Appendix (continued)

| Name | Location | Contribution |
|---------------------------------------|---|--|
| Martin Reuter, PhD | Image Analysis, German Center for Neurodegenerative Diseases (DZNE), Bonn; A.A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Boston; Department of Radiology, Harvard Medical School, Boston, MA | Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data |
| Monique M.B. Breteler, MD, PhD | Population Health Sciences, German Center for Neurodegenerative Diseases (DZNE), Bonn; Institute for Medical Biometry, Informatics and Epidemiology (IMBIE), Faculty of Medicine, University of Bonn, Germany | Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data |

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